

MBPCR166

Hi-PCR® Newcastle Disease Virus (NDV) Probe PCR Kit

Description

Newcastle disease virus (NDV) – A type strain for avian paramyxoviruses. Members of this family have a single-stranded, linear, RNA, with an elliptical symmetry. The total genome is roughly 16,000 nucleotides. NDV is a contagious and fatal viral disease affecting most species of birds. Clinical signs are extremely variable depending on the strain of virus, species and age of the bird, concurrent disease, and preexisting immunity. NDV is so virulent that many birds die without showing any clinical signs. A death rate of almost 100 percent can occur in unvaccinated poultry flocks. NDV can infect and cause death even in vaccinated poultry. NDV is spread primarily through direct contact between healthy birds and the bodily discharges of infected birds. Hence the diagnosis of such a virulent strain is of utmost importance to save the life of many birds.

NOTE: HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of NDV in animal samples.

Principle

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to

amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) Probe PCR Kit is designed to detect the gene specific to **NDV in FAM channel** and **Internal**

Control in JOE channel. The kit allows rapid, sensitive, and specific detection of NDV in a single tube reaction.

Negative Control

A negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, nuclease free water is used as the template.

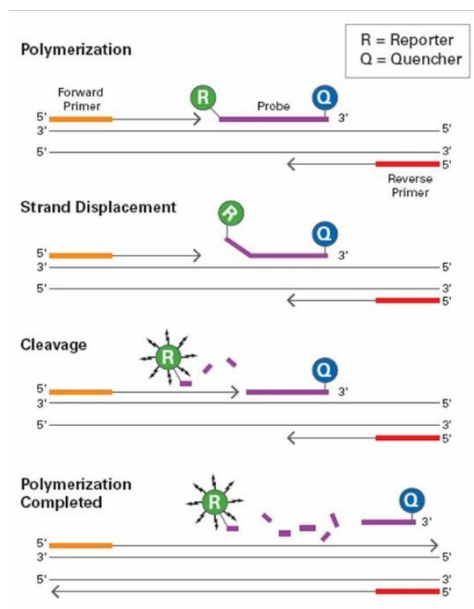
Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycle is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Fast and simple
- Good sensitivity and specificity
- Includes all reagents & controls for validity of the test

Sample Source: Viral culture/tissue from vital organs/clotted blood samples or serum

Specimen collection and Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred to in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

Kit Contents: The provided PCR kit contains

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
Hi-Quanti 5X Onestep Mastermix	MBT199	108	216
NDV Primer-Probe Mix	DS0392A	27	54
Internal Control Primer-Probe Mix	DS1021	27	54
Internal Control DNA	DS1022C	27	54
NDV Positive Control	DS0394A	30	60
Molecular Biology Grade Water for PCR	ML065	219	438

* For a 20 µL PCR reaction

Materials needed but not provided:

- All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96 [®] AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96 [®] AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96 [®] Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96 [®] - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96 [®] Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48 [®] M4 Real time PCR System, 96 well block, 4 channels	LA1023
Insta Q48 [®] M2 Real time PCR System, 96 well block, 2 channels	LA1024
TabSpin [®] Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX [®] Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX [®] Mag16, Insta NX [®] Mag16 ^{Plus}	LA1118, MBLA018
Insta NX [®] Mag32, Insta NX [®] Mag32 ^{Plus}	LA1096, MBLA019
Insta NX [®] Mag96	LA1097
Extraction Kits	
HiPurA [®] Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
HiPurA [®] Pre-filled Plates for Viral Nucleic Acid Purification	MB582MPF16
HiPurA [®] Pre-filled Plates for Viral Nucleic Acid Purification [For Insta NX [®] Mag32]	MB582MPF-32
HiPurA [®] Prefilled Plates for Viral Nucleic Acid Purification [For Insta NX [®] Mag96]	MB582MPF-96
HiPurA [®] Viral DNA/RNA Purification Kit	MB582
HiPurA [®] Multi-Sample DNA Purification Kit	MB554
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

Kit compatibility with Real-Time PCR Systems[#]

Hi-PCR[®] Newcastle Disease Virus (NDV) Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1	Dye 2
Insta Q96 [®] AG/ Insta Q96 [®] AG 6.0/Insta Q96 [®] - 6.0/Insta Q96 [®] Plus/Insta Q48 [®] M4	HiMedia Laboratories Pvt. Ltd.	FAM	HEX
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX
QuantStudio™ 5	Applied Biosystems	FAM	JOE/HEX/VIC
ABI [®] Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC
QIAquant 96 & 384 Splex	QIAGEN	FAM	JOE/HEX
Rotor-Gene [®] 6000 & Q	QIAGEN	FAM	JOE/HEX
LightCycler [®] 96 /LightCycler [®] 480	Roche	FAM	JOE/HEX/VIC
qTOWER ³	Analytik Jena	FAM	JOE/HEX/VIC

#Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufacturer's instructions and recommendations

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

Protocol for PCR Reaction Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), calculate the volume of the components to be added as N* volume of "1X"
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume to be added for 1R (for a 20 µL reaction)
Preparation of PCR Reaction Mix		
Hi-Quanti 5X Onestep Mastermix	MBT199	4 µL
NDV Primer-Probe Mix	DS0392A	1 µL
Internal Control Primer-Probe Mix	DS1021	1 µL
Internal Control DNA	DS1022C	1 µL
Molecular Biology Grade Water for PCR	ML065	7 µL
Total PCR Reaction Mix		14 µL
Template addition		
Template	-	6 µL
Total reaction volume	-	20 µL

4. Aliquot 14 µL of PCR reaction mix per reaction into 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add 6 µL per reaction of template into the plate/strip to respective wells.
6. For positive and negative control reactions, add 6 µL of NDV Positive Control and 6 µL of Molecular Biology Grade Water for PCR respectively. Refer the following table.

Set up of controls for the PCR run		
Components	Product code	Volume for "1X" (one reaction)
Total PCR Reaction Mix	-	14 µL
Positive Control		
NDV Positive Control	DS0394A	6 µL
Negative Control		
Molecular Biology Grade Water for PCR	ML065	6 µL
Total reaction volume	-	20 µL

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below).

Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

- | | | |
|--------------------------|------------------------------------|---------------------|
| 1. Reverse Transcription | : 55°C for 20 minutes | No. of cycles: 01 |
| 2. Initial denaturation | : 95°C for 03 minutes | No. of cycles: 01 |
| 3. Denaturation | : 95°C for 15 seconds | } No. of cycles: 40 |
| 4. Annealing | : 55°C for 15 seconds (Plate Read) | |
| 5. Plate Read | : FAM/ JOE | |

#for instruments not calibrated for JOE, HEX can be used

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

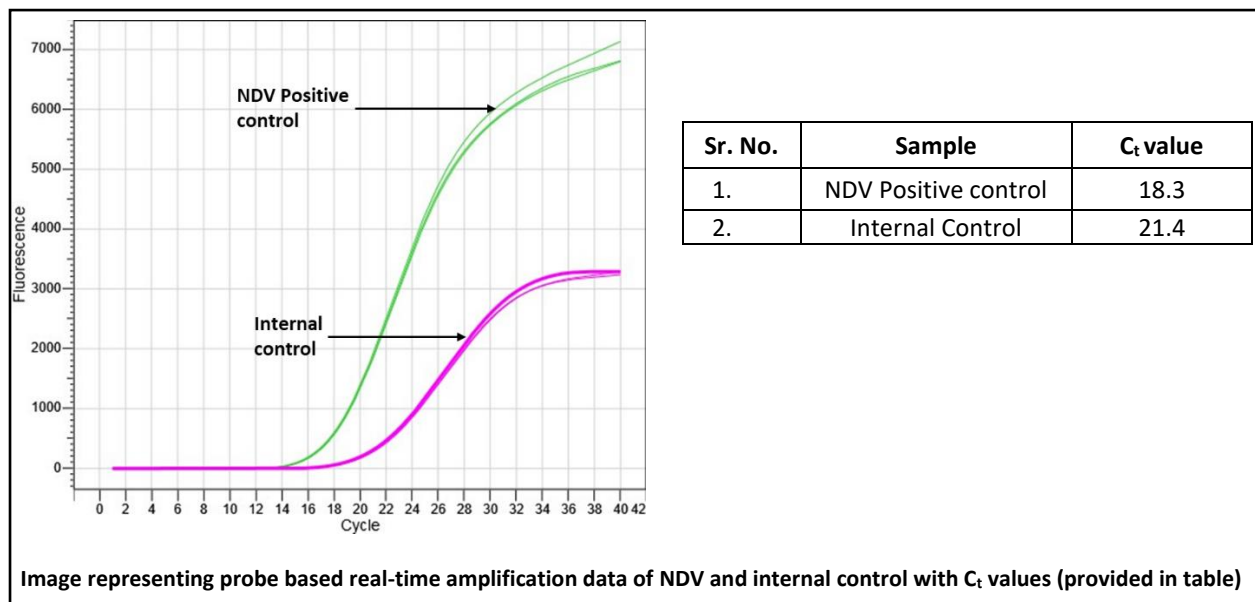
Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (New Castle Disease Virus)	JOE (Internal Control)
Positive Control	+	+/-*
Negative Control	-	+

*The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Amplification Data



Data Interpretation

Detection		Result Interpretation
FAM (New Castle Disease Virus)	JOE (Internal Control)	
+	+	Positive for New Castle Disease Virus
-	+	Negative for New Castle Disease Virus
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

C _t value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Positive result

Any sample that has a cycle threshold (C_t) value ≤ 40 associated with an amplification curve for target (FAM channel) is regarded as positive.

Any sample with C_t value > 40 should be reconfirmed by a repeat assay with fresh extraction of nucleic acid.

Negative result

Any sample that shows no C_t value and no amplification curve for target (FAM channel) and shows C_t value with amplification curve for internal control (JOE channel) is regarded as negative.

Performance Evaluation

Analytical Sensitivity - Limit of Detection (LoD)

The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability. The LoD assay for the Hi-PCR® Newcastle Disease Virus (NDV) was performed using Synthetic DNA by preliminary testing a 10-fold dilution series in triplicates per concentration followed by confirmation with 20 replicates of the concentration

determined to be the LoD. The detectable Limit of Detection (LoD) of NDV target was determined to be **5 copies/ μ L**.

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Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of nucleic acid using agarose gel electrophoresis. Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Check whether all components are added in correct volume as per the manual.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Incorrect PCR programming	Ensure selection of appropriate fluorescence channel as detailed in the manual. Compare the PCR program to the manual.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument.
		Pipetting error	C _t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes. Use calibrated pipettes. Repeat the run.
3.	Amplification of NDV target in negative control	Cross contamination during handling	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents. Follow good laboratory practices to avoid contamination issues.

Safety Information

HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) Probe PCR Kit is for laboratory use only, not for drug, household, or other uses. Take appropriate laboratory safety measures and wear gloves when handling.




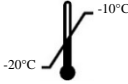




Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Following established laboratory procedures in disposing of infectious materials and materials that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBPCR166

Rev.No.:14

Date of Issue: 2025-06

Disclaimer :

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